Folia Neuropathologica

Mononuclear subsets in the peripheral blood of multiple sclerosis patients in relation to results of brain gadolinium – enhancing imaging

Grażyna Michałowska-Wender[1,2], Mieczysław Wender[2]

[1]Neurogenetic Unit, Medical University, Poznań, Poland; [2]Neuroimmunological Unit, M. Mossakowski Medical Research Centre, PASci, Poznań, Poland

Folia Neuropathol 2006; 44 (1): 67-71

Abstract

Very little is known about processes which are leading to the development of new multiple sclerosis (MS) plaques. Therefore, the problem of correlating immunological disequilibrium in MS patients with the onset of a relapse and with other markers of the disease seems to be of great importance. In our studies we have evaluated the mononuclear subsets in the peripheral blood of multiple sclerosis patients in relation to results of brain gadolinium-enhancing imaging. A positive magnetic resonance imaging (MRI) gadolinium enhancement indicates an ongoing inflammation and activity of the MS process, whereas gadolinium (Gd) negative imaging should be considered as a sign of stabilization of the MS process.

Material and methods: *Peripheral blood was taken from 70 MS patients, fulfilling the criteria of McDonald et al [8]. Brain MRI was performed using a Magneton Impact 1.0 T (Siemens) machine. Enhanced T1 images were obtained after administration of 0.1 mmol/kg gadolinium – DTPA. Lymphocyte subsets were analyzed by flow cytometry with the aid of specific monoclonal antibodies.*

Results: *The relative percentage of the white blood cell count as well as the absolute number of mononuclears and of CD3+ lymphocyte were significantly lowered only in the group of gadolinium negative MS cases. The CD4+ relative percentage was significantly higher both in the total as well as in gadolinium positive and negative subgroups. The ratio of CD4/CD8 was significantly higher in MS patients. The absolute number of CD4 lymphocytes was the lowest in gadolinium negative MS cases.*

Conclusion: *An appropriate regulation of the Th /T helper/ cells seems to be critical in the control and prevention of diverse states of the disease. In the course of an acute process, gadolinium positive imaging as well as immunological events, represented by mononuclear subsets in the peripheral blood may proceed and change very quickly, whereas gadolinium negative findings reflecting a stabilization of processes, are changing more slowly and therefore may be detected more easily in peripheral blood cell counts.*

Key words: *mononuclear subsets, multiple sclerosis, MRI, gadolinium enhancing.*

Communicating author:

Dr Grażyna Michałowska-Wender, Neuroimmunological Unit, Mossakowski Medical Research Centre, Przybyszewskiego 49, 60 –355 Poznań; tel. + 48 61 869 15 33, e-mail: mwender@am.poznan.pl

Introduction

Multiple sclerosis in thought to be an inflammatory T-cell mediated autoimmune disease characterized by inflammatory infiltrates. They are present in acute lesions in the central nervous system and are considered to be a prerequisite for the development of plaques and demyelination. Little is known about the first initiation stage of plaque formation, but the close contact of blood derived inflammatory cells with the brain endothelium via adhesion molecules is one of the most important and the earliest events to occur in this process.

T cells differ in their antigen recognition, have different functions and can cause various patho-physiological changes. Upon antigenic stimulation, they liberate various signal factors and initiate and amplify an immunological response. They also can be cytotoxic and exert a mediating effect by macrophage activation [12].

When compared with a healthy person, in multiple sclerosis (MS) there are multiple lines of evidence of significant changes in the pattern of mononuclear subsets of the peripheral blood [9]. Despite differences noted between various results of studies, it would appear that it is the significant increase of CD4+T cells and the higher CD4/CD8 ratios that constitute the so far established findings. The findings of Frequin et al. [3] who revealed in MS patients, especially in the chronic progressive group, significantly lower CD4+T cell percentages in the peripheral blood compared to counts seen in control specimens, are only a rare exception. However, considerable problems may arise when a precise definition changes in relation to different phases of the disease [1,4,5,11,15,16].

The only clinical delimitation of MS phases, based on the appearance of new symptoms indicative of a lesion in the central nervous system is inadequate. Hence the actual trend to preferably take into comparative consideration immunological findings and magnetic resonance imaging [6,14,18]. The problem of correlating immunological disequilibrium with the onset of a relapse and with other markers of the disease seems to be of great importance as well, because very little is known about processes which are leading to the development of new MS plaques.

In our present study we classified active and inactive MS stages by considering the magnetic resonance imaging (MRI) gadolinium enhancing. The positive MRI gadolinium enhancement indicates an ongoing inflammation and activity of the MS process, whereas gadolinium (Gd) negative imaging should be considered as a sign of stabilization of the MS process.

Material and methods

Peripheral blood was obtained once from 70 patients (46 females and 24 males) with clinically definite MS, fulfilling the criteria of Mc Donald at al. [8] and from 15 control healthy persons. According to the study protocol the blood was taken at the same time of the day to exclude diurnal variations of studied lymphocyte subsets. The MS patients were in the active relapsing – remitting phase of the disease, aged from19 to 46 years (mean 31 years), with relapses rate 1-2 in a year. Only two had three relapses in the year. None of the patients had an acute course of the disease. The mean duration of the disease was 30months /ranging from 10 to 38 month/. Mean EDSS was 2.7±0.9 (1.0 to 4.0). The patients had never received any disease modifying treatment (interferon beta, glatiramer acetate or any immunosuppressive drug) or any corticosteroids for at least 6 months prior to the onset of the study. The control group comprised 15 healthy adults, aged from 21 to 38 years.

The patients had brain MRI performed using a Magneton Impact 1,0 T (Siemens). T-1 proton-weighted, proton density, T2 – weighted spin echo, as well as gadolinium – DTPA - enhanced T1 images after administration of 0.1 mmol/kg gadolinium – DTPA, (Schering, Germany). 18 patients demonstrated brain gadolinium – enhancing lesions.

Lymphocyte subsets were analyzed by flow cytometry with the aid of specific monoclonal antibodies, a product of the Benton Dickinson Company. The evaluation of results was performed using the Cytoron Absolute (Ortho Diagnostic System). Readings were taken at a wavelength of 480 nm. The obtained relative results were evaluated using the Immuno Count II program.

For statistical analysis of results, the Starview statistical program was used. The Mann – Whitney U test was applied to determine the significance of differences between the studied groups.

Results

The relative percentage of the white blood cell count, as well as the absolute number of mononuclears were significantly lowered only in the group of

gadolinium negative MS cases. The absolute number of CD3+ lymphocytes was significantly lower only in gadolinium negative MS cases. Also the absolute number of CD3+ lymphocytes was significantly lower only in gadolinium negative cases. Compared to control values, the helper /inducer T lymphocyte /CD4+/ relative percentage was significantly higher both in the total as well as in either of the subgroups of MS cases (i.e. in gadolinium positive and negative subjects). Also the ratio of CD4/CD8 was significantly higher in MS patients (Table I). The absolute number of CD8 lymphocytes was the lowest in gadolinium negative MS cases. CD16 (macrophage monocytes) as well as CD19 (B cells) did not demonstrate differences in relation to statistically significant counts (Table 2). The singular results, which may be considered significant (difference from the mean of the control value, more than two times of the standard deviation) were observed only in very few cases.

Discussion

Multiple lines of evidence suggest that in multiple sclerosis, CD4 lymphocytes do initiate an autoimmune

Table I. Surface immunomarkers of the peripheral blood on mononuclear cells in patients with multiple sclerosis (MS) in relative %

	Number of patients	% of mononuclears in WBC count	CD_3	CD_4	CD_8	CD_4/CD_8	CD_{16}	CD_{19}
			in % to total lymphocytes					
MS total	70	31.53 ±15.46	80.66 ±6.93	50.39* ±11.24 p=0.033	32.03* ±14.26 p=0.030	1.81* ±0.81 p=0.03	11.20 ±15.87	13.84 ±15.47
MS gadolinium+	18	23.39 ±7.25 p=0.01	80.52 ±6.50	50.66* ±7.20 p=0.024	28.76 ±8.73	1.87* ±0.77 p=0.04	8.92 ±5.04	10.46 ±3.93
MS gadolinium-	35	28.66* ±10.66 p=0.04	79.71 ±5.31	50.16* ±6.72 p=0.007	28.82 ±6.84	2.01* ±0.98 p=0.02	8.64 ±3.28	11.66 ±3.68
control	15	35.27 ±9.95	80.47 ±7.79	44.00 ±7.41	34.38 ±9.51	1.41 ±0.56	8.06 ±5.03	11.48 ±5.91

*mean ± S.D. * differences significant MS patients/control*

Table II. Surface immunomarkers of peripheral blood mononuclear cells in patients with multiple sclerosis (MS) in absolute values (the number of cells in 1 µM)

	Number of patients	Mononuclears	CD_3	CD_4	CD_8	CD_{16}	CD_{19}
MS total	70	133.41 ±466.3	1073.6 ±392.6	647.9 ±248.6	406.1* ±179.7 p=0.03	117.0 ±0.42	152.2 ±77.0
MS gadolinium+	18	1395.9 ±345.7	1126.3 ±301.5	706.7 ±208.8	400.3 ±154.2	125.2 ±81.7	144.3 ±63.1
MS gadolinium-	35	1259.4* ±418.6 p=0.02	1006.8* ±344.4 p=0.02	624.9* ±245.2	363.0* ±113.6 p=0.07	106.4 ±48.3	146.1 ±68.6
control	15	1598.7 ±435.6	1295.7 ±397.6	703.8 ±232.0	554.4 ±212.7	120.1 ±62.0	182.9 ±106.9

*mean ± S.D. * differences significant MS patients/control*

response against the myelin antigen. The increased frequency of activated myelin specific cells in MS patients indicates that the reaction of autoreactive cells represents a central event in the pathomechanism of the disease. However, there are still significant controversies as to the correlation between MS relapses and deviations of T lymphocyte counts in the peripheral blood. Corrigan et al. [1] have established that the increased activity of the disease is not connected with changes of total T cell counts, total CD4+ cells, suppressor cells or activated T cells, but the relapse of MS happens to be accompanied by the conversion of CD4+ CD45R+ resting cells into CD4+ CD45R- primed cells. Gambi et al.[4] observed in MS patients an increase in the count of CD4+ CD29+ (helper–inducer cells), but this increase did not bear any relation to the different phases of the disease. Others authors [13] described a correlation of T lymphocyte subsets with the clinical activity of the disease, and Scolozzi et al. [16] found a positive correlation between CD4 T cells of both the C8F and peripheral blood with clinical fluctuation in MS patients. It seems to be undisputable that the T cells immune reaction broadens with time and thus is implicated in the progress of the pathological processes. However, no clear cut correlation has been found between the severity of the disease and T-cells immune responses to myelin epitopes [2].

The briefly presented differences between the results of the studies may be due to the fact that clinical observations are not well correlated with the occurrence of pathological events in the central nervous system. It is generally accepted that some lesions presenting in MRI images may appear without clinical manifestation, even at the time of remissions and the number of new lesions in the MRI picture - may be five to ten times higher that the number of relapses.

In our study we have classified the MS stages as active or inactive, by considering positive gadolinium (Gd) enhancement in MRI as the hallmark of active disease. Although there remains a considerable controversy with regard to the pathological background of Gd enhanced lesions in MRI, it is generally acknowledged that an enhancement is indicative of the presence of an ongoing acute inflammation in the central nervous system. Putheti et al. [14] have found a clear cut correlation between MRI parameters, including gadolinium – enhancing lesions on T1 – weighted images, chemokine receptor expression and the status of circulating /regulatory (CD4-CD25+) T cells/ Tr cells/. Similar results but concerning the cerebrospinal fluid (CSF) were obtained by Hui-Yun Wong et al. [6]. The number of GD-enhanced lesions has been shown to be correlated with CSF cells counts, as well as the number of CD4+CD29+/helper/inducer/ and Il-2 receptor /CD25+/ positive activated helper T cells. According to Killestein et al. [7] there is a positive correlation between the percentage of tumor necrosis factor-alpha-producing CD4(+) T cells and the change in T2 lesion load.

In our present studies we have been able to find that the absolute number of mononuclears, CD3+ and CD8+ was significantly decreased in gadolinium negative MS cases. Other lymphocyte markers under study, such as the relative percentage of helper/inducer T lymphocytes and the ratio of CD4/CD8 were found to be abnormal in both Gd positive and Gd negative cases. An appropriate regulation of the Th /T helper/ cells seems to be critical in the control and prevention of diverse states of the disease. These cells secrete soluble mediator molecules – the cytokines which orchestrate the immune response [10].

The explanation of our findings is not an easy one, therefore, first of all, the short-time evolution of autoreactive T cells repertoire in the active stage of MS should be taken into consideration [18]. The considerations may lead to the conclusion that in the course of an acute process, Gd positive imaging, as well as immunological events may proceed and change very quickly, whereas Gd negative findings, reflecting a stabilization of processes, are changing more slowly and therefore may be detected in peripheral blood cell counts. Some differences between the results of several published studies and also with our findings may be explained by the various methods of material classification. We have used for classification of cases not the clinical signs of a relapse but the results of MRI – imaging (gadolinium positive or negative findings), which was the case only in some studies of other authors.

References

1. Corrigan E, Hutchinson M, Feighery C. Fluctuations in T helper subpopulations in relapsing-remitting multiple sclerosis. Acta Neurol Scand 1990; 81: 443-447.
2. Davies S, Nicholson T, Laura M, Giovannoni G, Altmann DM. Spread of T lymphocyte immune responses to myelin epitopes

with duration of multiple sclerosis. J Neuropathol Exp Neurol 2005; 64: 371-377.
3. Frequin ST, Lamers KJ, Borm GF, Barkhof F, Jongen PJ, Hommes OR. T-cell subsets in the cerebrospinal fluid and peripheral blood of multiple sclerosis patients treated with high-dose intravenous methylprednisolone. Acta Neurol Scand 1993; 88: 80-86.
4. Gambi D, Porrini AM, Giampietro A, Macor S. CD21+ (B2 antigen+) cell decrement and CD4+CD29+ (helper-inducer) cell increment suggest an activation of cell immune reactivity in multiple sclerosis. J Neuroimmunol 1991; 33: 97-102.
5. Henneberg A, Fischer EG, Kornhuber HH. T – lymphocyte subpopulations in multiple sclerosis – do they help to judge immunosuppressive therapy? Eur Arch Psychiatry Neurol Sci 1988; 238: 94-96.
6. Wang HY, Matsui M, Saida T. Immunological disturbances in the central nervous system linked to MRI findings in multiple sclerosis. J Neuroimmunol 2002; 125: 149-154.
7. Killestein J, Kalkers NF, Meilof JF, Barkhof F, van Lier RA, Polman CH. TNF alfa production by CD4+ T cells predicts long-term increase in lesion load on MRI in MS. Neurology 2001; 57: 1129-1131.
8. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, McFarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, van den Noort S, Weinshenker BY, Wolinsky JS. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001; 50: 121-127.
9. Michałowska-Wender G, Losy J, Wender M, Januszkiewicz-Lewandowska D, Nowak J. Effect of immunomodulatory treatment of multiple sclerosis on lymphocyte surface immunomarkers. Pol J Pharmacol 2003; 55: 877-880.
10. Moss RB, Moll T, El-Kalay M, Kohne C, Soo Hoo W, Encinas J, Carlo DJ. Th1/Th2 cells in inflammatory disease states: therapeutic implications. Expert Opin Biol Ther 2004; 4: 1887-1896.
11. Munschauer FE, Stewart C, Jacobs L, Kaba S, Ghorishi Z, Greenberg SJ, Cookfair D. Circulating CD3+CD4+CD8+ T lymphocytes in multiple sclerosis. J Clin Immunol 1993; 13: 113-118.
12. Pichler WJ, Emmendorffer A, Peter HH, Deicher HR, Fontana A, de Weck AL. Analysis of T-cell subpopulations. Pathophysiological concept and significance for clinical medicine. Schweiz Med Wochenschr 1985; 115: 534-550.
13. Prencipe M, Salerni E, D'Aurizio C, Marini C, Adorno D, Berghella AM, Pellegrini P, Papola F, Casciani CU. T – lymphocyte subsets modifications in multiple sclerosis: correlation with clinical disease activity. Ital J Neurol Sci 1986; 7: 583-587.
14. Putheti P, Morris M, Stawiarz L, Teleshova N, Kivisakk P, Pashenkov M, Kouwenhoven M, Wiberg MK, Bronge L, Huang YM, Soderstrom M, Hillert J, Link H. Multiple sclerosis: a study of chemokine receptors and regulatory T cells in relation to MRI variables. Eur J Neurol 2003; 10: 529-535.
15. Salmaggi A, Bianchi G, Cerrato D, Lazzaroni M, Malesani L, Nespolo A, Corridori F, La Mantia L, Milanese C. Cerebrospinal fluid and peripheral blood T-lymphocyte subsets in multiple sclerosis: monoclonal antibody analysis and correlations with clinical activity. Ital J Neurol Sci 1987; 8: 327-330.
16. Scolozzi R, Boccafogli A, Tola MR, Vicentini L, Camerani A, Degani D, Granieri E, Caniatti L, Paolino E. T – cell phenotypic profiles in the cerebrospinal fluid and peripheral blood of multiple sclerosis patients. J Neurol Sci 1992; 108: 93-98.
17. Skulina C, Schmidt S, Dornmair K, Babbe H, Roers A, Rajewsky K, Wekerle H, Hohlfeld R, Goebels N. Multiple sclerosis: brain-infiltrating CD8+T cells persist as clonal expansions in the cerebrospinal fluid and blood. Proc Natl Acad Sci USA 2004; 101: 2428-2433.
18. Vergelli M, Mazzanti B, Traggiai E, Biagioli T, Ballerini C, Parigi A, Konse A, Pellicano G, Massacesi L. Short-term evolution of autoreactive T cell repertoire in multiple sclerosis. J Neurosci Res 2001; 66: 517-524.